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Picoplankton diversity in the South-East Pacific Ocean from cultures

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Abstract

In late 2004, the BIOSOPE cruise sailed between the equatorial influenced waters off Marquesas islands and the nutrient enriched waters of the Chilean upwelling. Along the way, it explored the Southeast Pacific gyre centred around Easter Island, which is probably the most oligotrophic oceanic region on earth. During this cruise, we undertook a vigorous effort to isolate novel photosynthetic picoplanktonic eukaryotes. Two strategies were attempted on board: enrichment of samples with culture medium and sorting of specific populations by flow cytometry based on chlorophyll fluorescence. Over 1900 pre-cultures were started and then further purified by flow cytometry, serial dilution or pipette isolation to yield a total of 212 strains. These strains were characterized morphologically and for more than 50% of them, genetically, through partial sequencing of the 18 S rRNA gene.

Among the characterized strains, the largest number are stramenopiles (Heterokontophyta) with a record of 38 strains belonging to the species *Pelagomonas calceolata* (Pelagophyceae). Strains from the recently described genera *Bolidomonas* and *Florenciella* have been re-isolated for the first time since their description. Two other abundant groups are the Chlorophyta, especially Prasinophyceae, and the Haptophyta, especially the genera *Phaeocystis* and *Emiliania*. A limited number of heterotrophic flagellates have also been isolated, all of them closely related to known species. Finally over a dozen of unicellular cyanobacteria strains have been obtained, some forming unusual short chains.

Overall our strategy was quite successful since it allowed us to isolate a large number of picoplankton strains but failed in two respects. First, apparently very few novel taxa have been obtained. One set of strains is related to *Prasinoderma coloniale* (Prasinococcales, Prasinophyceae) but their sequences are sufficiently different from the latter to probably belong to a new genus or species. The sequences of two other strains are phylogenetically affiliated to stramenopile environmental sequences, probably corresponding a new algal class. Second, very few strains have been obtained

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from the very oligotrophic central gyre itself. Future work should probably combine flow cytometry sorting with culture media and cultivation approaches specifically developed for oligotrophic water species.

1 Introduction

Thirty years ago, the existence of very small algal cells was discovered in marine waters (Johnson and Sieburth, 1982; Waterbury et al., 1979). They were termed picoplankton, defined as smaller than 2–3 μm (Sieburth et al., 1978). It was soon realized that a significant fraction of photosynthetic biomass and primary production could be attributed to these tiny cells (Li et al., 1983; Platt et al., 1983). This small size fraction was found to be more important as chlorophyll concentration decreased, i.e. as the degree of oligotrophy increased (Herbland et al., 1985). Within photosynthetic picoplankton, prokaryotes appeared early on as much less diversified than eukaryotes as they are dominated by two major cyanobacteria genera: *Prochlorococcus* and *Synechococcus*. This probably explains why we now know much more about photosynthetic picoplanktonic prokaryotes than eukaryotes. In particular, the genetic diversity of these prokaryotes has been quite well characterized (Fuller et al., 2003; Rocap et al., 2002), representatives of key genotypes have been isolated in culture, and more recently quite a few genomes have been sequenced (Palenik et al., 2003; Rocap et al., 2003). It is now possible to map the distribution of key groups of cyanobacteria in oceanic waters and to assess the existing relationships between genotypes and ecotypes (Johnson et al., 2006).

For photosynthetic picoeukaryotes, the situation is, in many respects, much less advanced, one reason being their very wide phylogenetic diversity. They belong to at least four major lineages: Chlorophyta, Haptophyta, stramenopiles (or Heterokontophyta) and Alveolata. Moreover, extensive studies of their genetic diversity from environmental samples only started less than 10 years ago (López-García et al., 2001; Moon-van der Staay et al., 2001). To date, about 20 species that are picoplanktonic

sensu stricto (i.e. for which cells are always smaller than 3 μm) have been described (Vaulot et al., 2007[1]). Among these, knowledge about "flagship" species such as *Ostreococcus* and *Micromonas* (both belonging to the order Mamiellales, Prasinophyceae) is progressing fast since the genome of several "ecotypes" has already been (or is currently) sequenced (Derelle et al., 2006; Palenik et al., 2007). Their oceanic distribution can be mapped using techniques such as fluorescent in situ hybridization (Not et al., 2005) or quantitative PCR (Marie et al., 2006). However, this only constitutes the tip of the iceberg as molecular approaches, in particular the analysis of 18S rDNA genetic libraries from the natural environment, have pointed out to a very wide diversity at all taxonomic levels (Vaulot et al., 2007[1]). For example, a new division of photosynthetic eukaryotes, the picobiliphytes, has been recently discovered (Not et al., 2007). At the class, order or genus level many taxa are only known from their sequences. This is the case for example for Prasinophyceae clade VII B (Guillou et al., 2004) or for *Chrysochromulina*-related clades within the prymnesiophytes (Moon-van der Staay et al., 2000). For all these taxonomic groups, there is a critical need to obtain cultured representatives. This concern is especially acute in open ocean oligotrophic regions due to the difficulty to isolate and maintain organisms adapted to low nutrient conditions that are often outgrown by fast dividing "weed" species.

The BIOSOPE cruise that sailed through the center of the South East Pacific gyre, probably the most oligotrophic place on earth, offered an opportunity to obtain cultures from this unique environment. We performed sample enrichment with diluted culture medium following filtration to separate the smaller picoplankton cells from the rest of the plankton, a strategy that allowed us in the past to obtain novel taxa (Vaulot et al., 2004). We also targeted specifically photosynthetic picoeukaryotes by using flow cytometry sorting directly on board the ship. In the end, we obtained 212 cultures that have been integrated to the Roscoff Culture Collection (RCC), more than half of which were characterized genetically by sequencing partially the 18S rRNA gene. These cul-

[1]Vaulot, D., Eikrem, W., Viprey, M., and Moreau, H.: The diversity of eukaryotic marine picophytoplankton, FEMS Microbiol. Rev., submitted, 2007.

tures encompass representatives of five major phylogenetic divisions: Cyanobacteria, Chlorophyta (mostly Prasinophyceae), stramenopiles, Haptophyta, Alveolata (dinoflagellates), Euglenozoa (bodonids).

2 Material and methods

2.1 Sampling

Samples were taken in general at two depths (surface layer and vicinity of the chlorophyll maximum) at selected stations along the BIOSOPE cruise track (Fig. 1 and Table 1) using Niskin bottles mounted on a CTD frame. The oceanographic context of the cruise is described in Claustre et al. (2007)[2].

2.2 Primary cultures

We used two different strategies to obtain starter cultures. The first one was based on filtered seawater enriched with nutrients. The second one relied on single cell sorting by flow cytometry, targeting specific cell populations based on their size and pigment fluorescence. As cultures were examined several times during the cruise, many variations were attempted in an effort to increase final culture yield.

2.2.1 Growth conditions used on board

All cultures were incubated on board in a thermostatic cabinet set at 20°C. Two light levels were obtained with 2 Sylvania 18 W tubes: white light around 140 μmol photons.m^{-2} s^{-1} and blue light (Moon Light Blue paper, M.E.S, Nantes, France) around 8 μmol photons m^{-2} s^{-1}. We used three types of medium: K (Keller et al., 1987)

[2]Claustre, H., Sciandra, A., Vaulot, D., and Raimbault, P.: Introduction to the special section: bio-optical and biogeochemical conditions in the South East Pacific in late 2004 – the BIOSOPE program, Biogeosciences Discuss., in preparation, 2007.

for photosynthetic eukaryotes, Pro2 (Moore and Chisholm, 1999) for photosynthetic prokaryotes (*Prochlorococcus* and *Synechococcus*), and rice-based (Massana et al., 2004) for heterotrophic eukaryotes which were grown in the dark. Multi-well plates were wrapped with parafilm in order to avoid any evaporation during growth.

2.2.2 Enrichment cultures

About 500 mL of sample seawater was filtered by simple gravity through two superposed (in an effort to provide more tight size fractionation) Nuclepore filters of 47 mm diameter, with either 0.6 μm or 3 μm porosity (Whatman International Ltd, Maidstone, UK). The filtrate was partitioned into 50 mL culture flasks (Sarstedt, Orsay, France) or, at one station (HNL3), into individual wells of 24-well plates to which we added either 1/10 or 1/100 of full strength K or Pro2 medium. In order to try to promote nitrogen fixing organisms, some cultures were started by simply amending sea water with iron (as $FeCl_3$) and phosphorus (as KH_2PO_4 at final concentrations of 3 nM and 0.4 μM, respectively.

2.2.3 Cultures sorted by flow cytometry

Samples were run either un-concentrated or concentrated between 5 and 100-fold by tangential flow filtration using a 100 000 MWCO (Regenerated Cellulose – RC ref VF20C4) Vivaflow 200 cassette. Concentration was usually necessary to target the rarer cells. Between 1 to 500 000 cells were sorted using a FACSAria (Becton Dickinson, San Jose CA) flow cytometer either into 24 or 48-well plates or directly into 10 mL polystyrene tubes pre-filled with medium diluted 100 times (Table 2). Different cell populations were discriminated based on side scatter as well as orange and red fluorescence following excitation at 488 nm (20 mW) and sorting was done either in purity or yield mode.

2.3 Primary culture processing and establishment of strains

On board the ship, primary cultures (either enriched or flow sorted) were checked for growth once or twice (depending on how early in the cruise they were started) using flow cytometry and inverted microscopy. Cultures that displayed growth but appeared mixed were sorted a second time.

A first set of cultures were transferred back to Roscoff on the occasion of change of crew at Easter Island at mid-cruise. At the end of the cruise, cultures from the early part of the cruise (i.e. about two months old) that showed no evidence of containing photosynthetic cells based on flow cytometry analysis were discarded. All cultures grown in multi-well plates were transferred to 10 mL polystyrene tubes and brought back to Roscoff in an ice box.

Once transferred back to Roscoff, cultures were monitored based on colour as well as with optical microscopy and flow cytometry. Cultures were purified either by serial dilution, solid medium plating, or individual cell pipetting under an inverted microscope. Strains that appeared to be pure were transferred to normal strength medium [PCR-S11 (Rippka et al., 2000), K, and rice for cyanobacteria, autotrophic and heterotrophic eukaryotes, respectively] and entered into the Roscoff Culture Collection (RCC) under new accession numbers (Table 2).

2.4 Strain characterization

Strains deposited to the RCC were characterized by optical microscopy. For each strain, pictures were taken on live cultures with an Olympus BX51 microscope with a x100 objective using differential interference contrast (DIC) with a SPOT RT-slider digital camera (Diagnostics Instruments, Sterling Heights, MI). Average cell dimension of each culture was determined from the pictures. Flagellated cells were also photographed after adding one drop of lugol to visualize flagellum shape, length and number. Cyanobacteria were identified by their colour and shape. The morphology of a few strains was confirmed by whole-mount transmission electron microscopy. Cells

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were fixed for 15 min with 1% glutaraldehyde. A drop of fixed cells was deposited onto formvar-coated grids. When the drop had dried, grids were rinsed with distilled water. Cells on grids were stained with a saturated solution of uranyl acetate for 20 min and rinsed with distilled water. Photomicrographs were taken with a JEOL JEM-1200EX electron microscope.

A subset of strains was characterized by their partial 18 S ribosomal RNA gene sequence. Cultures were grown in 50 mL flasks for 1–2 weeks depending on the growth of each strain and recovered by centrifugation at 11 000×g for 10 min. DNA was extracted using 3% Cethyl Trimethyl Ammonium Bromide (CTAB, Doyle and Doyle, 1990). DNA was then stored at –80°C.

The 18S rRNA gene was amplified by polymerase chain reaction (PCR) using the primer set Euk328f and Euk329r (Moon-van der Staay et al., 2000) and the HotStarTaq Master Mix (Qiagen, Courtaboeuf, France). For PCR, a 15 min initial activation step of the Polymerase at 95° C, was followed by 40 cycles including 1 min of denaturation at 94° C, 45 s of annealing at 57° C and 75 s extension at 72° C. The PCR program was finished by a final extension of 10 min at 72° C followed by cooling at 4° C. PCR products were purified with the Qiaquick PCR purification kit (Qiagen) and controlled by electrophoresis on a 1% agarose gel. Partial 18 S rRNA gene sequences were determined from purified PCR products by using Big Dye Terminator V3.1 (Applied Biosystems, Foster city, CA, USA) and the internal primer Euk 528f (Elwood et al., 1985) run on an ABI prism 3100 sequencer (Applied Biosystems, Courtaboeuf, France).

Sequences were compared to those available in public database with NCBI BLAST web application. Sequences were also automatically aligned using the ARB program (Ludwig et al., 2004) to a set of more than 20 000 high quality pre-aligned eukaryotic sequences available from the Silva database (database SSURef: http://www.arb-silva.de). After manual refinement of the alignment, sequences were added to the reference tree provided with the SSURef database using the quick parsimony addition option. Sequences with high similarities were grouped together using Fast Group II (http://biome.sdsu.edu/fastgroup/fg_tools.htm) with the sequence match pa-

rameter set at 80 % and one or two representative sequences per group were chosen along with the closest publicly available sequence. Phylogeny analysis was performed on aligned sequences with MEGA4 (http://www.megasoftware.net/, Tamura et al., 2007). A neighbour-joining tree was computed from 394 common positions based on Kimura 2-parameter model distances using 1000 bootstrap replications. Sequences have been submitted to GenBank under accession number xxx-xxx.

3 Results

3.1 Isolation success

All together more than 1900 starter cultures were established during the BIOSOPE cruise (Table 1) either as enrichment cultures following filtration through either 0.6 or 3 μm or by sorting specific populations into individual wells or tubes. From one to three purification steps were in general necessary to obtain pure cultures (Table 2). For example, enrichment cultures started at the beginning of the cruise were sorted at the end of the cruise and then purified by serial dilution back in the laboratory.

In the end, we obtained 188 autotrophic and 24 heterotrophic cultures which have been deposited to the RCC (Table 2). Among these, 12 were subsequently lost and 25 remain not pure to this date. The latter are mostly autotrophic strains contaminated by heterotrophic eukaryotes. Cruise coverage was quite unequal with many strains obtained in mesotrophic regions and in the Chilean upwelling and much fewer from the central gyre (Table 2, Fig. 1). This reflects probably the difficulty to obtain cultures representative of extreme oligotrophic conditions, since nutrient additions even at relatively low concentrations are always much higher than those found in the environment. However this unbalanced coverage is not only the consequence of the environment but also of practical considerations. Cultures started early during the cruise had a chance to be screened before the end of the cruise and therefore could be re-purified on-board the ship. Conversely, cultures started late in the cruise were transported during their initial

growth phase, a period where they may be less fragile than once they have acclimated to more stable conditions. Refinements in culturing conditions that were implemented late in the cruise based upon results obtained in the first part of the cruise may also explain why our success rate was good by the end of the cruise. For example, starter cultures were sorted at the beginning of the cruise into 24 or 48-well plates. By mid-cruise, as we did not observe any growth under these conditions, we decided to switch to sorting into 10-mL tubes which seemed to result in a higher success rate.

Sorting was an important element since more than 65% of the final cultures had undergone a sorting step. The strategy that yielded most pure strains was first to establish an enrichment culture with either 0.6 or 3 μm filtered samples followed by sorting sometimes later. In this case, it was often not necessary to perform further purification by serial dilution, saving this labour-intensive step. Sorting directly from natural samples was rarely sufficient to produce pure cultures and in most cases a second purification step had to be undertaken. It is difficult to determine whether sorting was successful in isolating the initially targeted population. We sorted sub-populations on the base of side scatter and chlorophyll but each of these sub-populations does not appear to be uniform genetically and consists probably of a mixture of several taxa belonging to different algal classes (Shi, X. and Marie, D., unpublished).

3.2 Culture diversity

All purified cultures were examined by light microscopy, imaged digitally and their average size was determined (Table 2 and Fig. 2). No attempts were made to record measurements for a large number of cells in each cultures and these data are therefore only indicative. They confirm, however, that our efforts to target picoplankton were successful since the mode size for the culture set lies between 2.5 and 3 μm.

A large, randomly chosen, subset of cultures (115, Table 2, Table 3) was analysed phylogenetically by sequencing either partially or, in a few cases, totally the 18 S rRNA gene. A few other cultures were identified based on their phenotypic characteristics (cyanobacteria, microplanktonic species).

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Representatives of cyanobacteria and of three major eukaryotic divisions containing photosynthetic organisms (stramenopiles, Chlorophyta, and Haptophyta) have been obtained in culture with the former most prevalent and the latter two in almost equal proportions (Table 4). Two dinoflagellate cultures have also been isolated.

Thirteen strains of unicellular rod-shaped cyanobacteria have been obtained tentatively identified as *Synechococcus*. No *Prochlorococcus* was obtained despite the use of the *Prochlorococcus* specific Pro2 medium. Some of these cyanobacterial strains form short chains, exhibiting sometimes very elongated cells (Fig. 3, RCC 1027) contrasting the usual *Synechococcus* morphology (Fig. 3, RCC 1022). Such strains mostly originated from the HNLC station near the Marquesas islands (Table 3) and could be interesting since samples from this region displayed an unusually high fraction of chain-forming and colonial picocyanobacteria (Masquelier and Vaulot, 2007). Phylogenetic analyses of the 16 S rRNA gene will be necessary to determine the exact nature of these strains.

Chlorophyta, and more specifically Prasinophyceae, are important contributors to picoplankton and many strains have been isolated from marine waters in the past, some of them belonging to not yet described species (Guillou et al., 2004; Vaulot et al., 2004). Seventeen Chlorophyta strains have been isolated during BIOSOPE, mostly Prasinophyceae. Among these, 11 are related to *Prasinoderma coloniale* (Prasinococcales), a picoplanktonic species that can form colonies surrounded by mucus. These strains display the bilobed cup-shaped chloroplasts characteristics of *P. coloniale* (Hasegawa et al., 1996). However most of our strains do not seem to form colonies as *P. coloniale* does. Interestingly, one group of 9 sequences appear to form a separate clade (Fig. 4) with only 94.7% identity to *P. coloniale* (in contrast to the two other strains sharing 99.6% identity with *P. coloniale*) and possess large and highly similar insertions at least 330 bp long inside de 18 S rRNA gene starting at nucleotide position 862 of the *P. coloniale* sequence. Phenotypically, strains from these group appear slightly smaller (Table 2) than those closely related to *P. coloniale*. They were isolated from near-surface waters at a variety of stations, while the two strains more closely related to *P. coloniale*

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originated from the Marquesas area. A culture closely related to *Prasinococcus capsulatus*, a species that also belongs to the order Prasinococcales, has been recovered from the chlorophyll maximum at the GYR station. Cells display a polysaccharide capsule around the cell (Fig. 3, RCC 859), typical of this species (Miyashita et al., 1993). Five Prasinophyceae closely related to the picoplanktonic species *Pycnococcus provasolii* (Pseudoscourfieldiales) have been isolated from two mid-depth samples in the Chilean upwelling. Eight strains belong to clade VII A of the Prasinophyceae (Guillou et al., 2004), a group which contains some cultured strains such as CCMP 1205 but for which no species has been described formally. All these strains consist of small (2 to 4 μm) coccoid cells lacking discriminating features (Fig. 3, RCC 857). Two sets of strains originated from surface waters and one set from 100 m in the HNLC zone. Two Prasinophyceae strains from the Chilean upwelling belong to clade C of the very ubiquitous species *Micromonas pusilla* (Guillou et al., 2004). They possess an unusually long flagellum (Fig. 3, RCC 913) that could be a diagnostic feature for that clade (Jouenne, F., personal communication). Finally, one culture (RCC 999) presents some phylogenetic affinities to the Prasinophyceae but its partial 18 S rDNA sequence does not allow us to place it in any of the existing clades defined by Guillou et al. (2004). We also isolated from one sample of the Chilean upwelling, three cultures representative of another green algal class, the Trebouxiophyceae. These strains are phylogenetically related to the recently established genus *Picochlorum* (Fig. 4) that regroups now salt-tolerant *Nanochlorum* (Henley et al., 2004).

All Haptophyta cultures are part of the class Prymnesiophyceae. Sixteen strains belong to the genus *Phaeocystis*, three from the upwelling region being more closely related to the species *P. jahnii* which has been recently described from the Mediterranean Sea (Zingone et al., 1999) and forms loose colonies and 5 from the east of the gyre and upwelling regions related to *P. globosa* that forms spherical colonies (Fig. 3, RCC 851). We also isolated 12 strains of *Emiliania huxleyi*, a few calcifying (Fig. 3, RCC 867) and most naked (Fig. 3, RCC 951), corresponding probably to diploid and haploid stages, respectively (Houdan et al., 2003). Two other unidentified coccolithophorids have also

been obtained from the Marquesas and central gyre regions. Interestingly all Haptophyta strains were isolated from the top of euphotic zone (between 5 and 60 m).

Among stramenopiles, 38 cultures are closely related to the picoplanktonic species *Pelagomonas calceolata* (Pelagophyceae). More than half of them are flagellated (Fig. 3, RCC 879), fitting the original description of the species (Andersen et al., 1993), and the rest, coccoid. However, the presence of a flagellum reflects probably more life cycle stages rather than taxonomical differences since both flagellated and non-flagellated strains have been isolated from the same sample (e.g. at 100 m at the HLN station). The presence of a thin theca characteristics of the species (Andersen et al., 1993) was confirmed by electron microscopy on strain RCC 879. *P. calceolata* has been isolated at a variety of stations (Marquesas, HLNC, center of gyre, east of gyre and upwelling) both in surface and at 100 m, demonstrating that this species is truly ubiquitous in oceanic waters. Interestingly in the center of the South East gyre, *Pelagomonas* strains were isolated from very deep samples down to 160 m. Two Pelagophyceae strains (RCC 986 and 1024) with 18 S rDNA sequences displaying slightly lower similarity to *P. calceolata* (Fig. 4) were recovered at 60 m depth from the Marquesas region. Both are picoplanktonic and coccoid, not displaying any specific morphological features. We isolated a novel strain with high similarity to *Bolidomonas pacifica*, a species that belongs to the recently described class of the Bolidophyceae (Guillou et al., 1999), closely related to the diatoms. Its morphology (presence of 2 unequal flagella) was confirmed by electron microscopy. This is quite interesting since to our knowledge this is the first novel isolate from this class since its initial discovery. In the same manner, we isolated from Marquesas surface waters, two strains very closely related by their 18 S rDNA sequence to the recently described Dictyochophyceae picoplanktonic species *Florenciella parvula* (Eikrem et al., 2004). Similarity was also confirmed by electron microscopy. Two photosynthetic stramenopile strains could not be assigned to any specific class as they shared homology with both *Pinguiococcus* (Pinguiophyceae) and *Nannochloropsis* (Eustigmatophyceae). Their 18 S sequences are almost identical to an environmental 18 S sequence (BL000921.5) recovered from

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Blanes Bay in the Mediterranean Sea (Fig. 4). They could belong to a new class, although the presence of refractive intracellular granules (Fig. 3, RCC 853) is quite reminiscent of what is observed in *Nannochloropsis*. Unfortunately, they have been lost in early 2007 following a breakdown in the air conditioning system of our culture facility. Their loss, which was almost the only one from a quite large collection, attests of their sensitivity to change in environmental conditions and may explain why representatives of this group have not been isolated before.

Three diatoms, belonging to the genera *Chaetoceros* (Fig. 3, RCC 1025) *Thalassiosira*, and *Minutocellus* were obtained from the upwelling region. The latter strain is quite interesting since its very small size (about 3 μm) connects it to picoplankton. Two dinoflagellates belonging to the genus *Prorocentrum, P. minimum* (Fig. 3, RCC 922) and *P. dentatum*, were isolated from surface waters, east of the gyre.

Twelve heterotrophic strains from dark cultures growing on rice medium have been identified by their 18 S rRNA sequences. Nine belong to the bicosoecid lineage of the stramenopiles. Three cultures are quite closely related to the genus *Caecitellus* and four more distantly related to *Cafeteria*. The two remaining strains were closely related to the bodonid (Euglenozoa) genera *Rhynchomonas* and *Neobodo*. All these genera are quite often recovered in cultures (Arndt et al., 2003).

4 Conclusions

Our large scale effort to isolate picoplanktonic strains from the Southeast Pacific Ocean allowed us to obtain of 212 novel cultures, a large number of which are of picoplanktonic size. The final number of cultures obtained is substantially higher than in previous efforts such as those linked to the PROSOPE and MINOS cruise in the Mediterranean Sea or the OLIPAC cruise in the Equatorial Pacific Ocean from which we obtained between 46 and 90 strains for each (Vaulot et al., 2004). Our initial intent was to use mostly flow cytometry sorting to establish this strains. However as we experienced technical problems with flow cytometry in the first few days of the cruise and as

we observed subsequently that the yield of the initially sorted samples was quite low, we decided to combine flow cytometry sorting with more classical enrichments. This proved to be quite a good recipe, especially since sorting based on photosynthetic pigment fluorescence appears as a good way to prevent contamination of cultures by heterotrophic eukaryotes, a problem plaguing some of our previous efforts. The application of sorting either before or after enrichment did not appear to affect dramatically the type of taxa isolated (Table 2).

The final diversity achieved is quite wide since we obtained representatives of most major photosynthetic divisions (Table 4). However it is clear that we globally failed to obtain representatives of environmental sequences for which no culture is available yet. One interesting group of novel cultures was constituted by stramenopile strains RCC 853 and 862 from the central gyre which sequences were closely related to an environmental sequence from the Mediterranean Sea (Fig. 4). Although these sequences had some affinities, based on BLAST, to Eustigmatophyceae and their morphology was somewhat similar to the latter, they probably belonged to a novel class. Despite the fact that further studies are prevented since these strains have been lost, the strategy used (flow cytometry sorting followed by serial dilution) could be tried again to re-isolate them. Another interesting group is constituted by 9 cultures originating from the region east of the gyre and from the upwelling that are related to *Prasinoderma* but form a new clade clearly separated from the species *P. coloniale* (Fig. 4). They could belong to a new species within the genus *Prasinoderma* or form a new genus. Interestingly, they are apparently not related to any published environmental sequence. All the other cultures obtained are related to described species or at least to established cultures. In particular, we have been successful at re-isolating two genera *Bolidomonas* and *Florenciella* that our group had previously isolated and described (Eikrem et al., 2004; Guillou et al., 1999), but that had never been obtained again in culture since their initial isolation. Interestingly, *B. pacifica* was initially isolated in exactly the same region (between 2 and 16° S) as the new strain (9° S). In contrast, the only *F. parvula* strain available previously originated from English Channel coastal waters, a very different

environment from that of the new strains. Moreover the 18S sequences of the latter differ slightly from that of *F. parvula* and they could belong to a novel species within this genus. Some of the cultures recovered correspond to ubiquitous species that were obtained from a wide range of environments. This is in particular the case for the two Haptophyta genera *Emiliania*, isolated from two of the four major regions investigated (Marquesas, east of gyre) and *Phaeocystis* isolated from three regions (Marquesas, east of the gyre, Chilean upwelling) mostly in surface waters. For the latter genus, our strains may correspond to at least two different species, *P. globosa* and *P. jahnii*. However, the largest number of strains obtained for a single taxon correspond to *Pelagomonas* isolated from a record of 13 different samples along the entire cruise track ranging from oligotrophic (St B13) to eutrophic (UPX) and from surface (5 m) to very deep (160 m) samples. Although the similarity of their 18S rRNA gene sequence is very high (average p-distance=0.0018), it is likely that these strains present quite different growth responses to factors such as nitrogen supply or light levels and belong to different ecotypes, as observed previously for example for the genus *Ostreococcus* (Rodríguez et al., 2005).

From a biogeographic point of view, it is quite difficult to make any firm conclusion from this work. Many cultures belonging to a given taxonomic group were isolated from a variety of conditions and no specific pattern could be uncovered. Although there were some taxa unique to the central part of the gyre itself (stations 3 to 15) such as *Prasinococcus* and the potentially novel class mentioned earlier, one should emphasize the low number of strains isolated from this region. This is probably linked to the fact that the media we used (K, Pro2), that are quite successful in general to isolate and maintain a wide variety of picophytoplankton strains, fail to mimic the drastic oligotrophic conditions met in the gyre. Moreover future isolation effort may need to involve new culture approaches such as those successful to isolate fastidiously growing bacterial strains from the open ocean environment such as *Peligibacter ubique* (aka SAR11) that had escaped cultivation for quite a long time (Zengler et al., 2002).

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Table 1. Sampling stations and number of starter cultures for each station. Depths are expressed in meters.

Station	Depth min	Depth max	Filtration <0.6 μm	Filtration <3 μm	Sort
SE3	15	70			288
MAR	10	60	10	20	288
HLN	30	100	48	48	96
STB2	30	100			192
STB4	40	140			192
STB7	5	175			240
GYR2	5	500		8	72
STB11	0	200		12	48
STB12	40	180			48
STB13	0	160		16	
STB14	5	150			72
STB15	100	100			48
EGY2	5	80	32		112
STB17	0	20			19
STB20	5	45	16		8
STB21	5	5			4
UPW1	5	35	16		20
UPX	0	40	16		7

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Table 2. List of strains deposited to the Roscoff Culture Collection (RCC) ordered according to the sampled station. Steps 1, 2 and 3 refer to the different purification steps performed before the culture was entered to the RCC database.

RCC	Lost	Station	CTD	Depth	Preculture	Class	Genus	Size	Mixed	Hetereo-trophic	Step 1	Step 2	Step 3	Sorting target	Step 1 medium
923		MAR3	22	10	273_FL1-2	Unknown	Unknown	2.5	+		TFF enriched	Serial dilution			None
959		MAR3	22	10	273_FL2-2	Unknown	Unknown	2.5			TFF enriched	Serial dilution			None
960		MAR3	22	10	273_FL2-3	Unknown	Unknown	3			TFF enriched	Serial dilution			None
961		MAR3	22	10	273_FL2-7	Unknown	Unknown	3			TFF enriched	Serial dilution			None
907		MAR3	22	60	271_FL1-4	Prasinophyceae	*Prasinoderma*	3.5			TFF enriched	Serial dilution			None
1000		MAR4	28	10	30 A	Prymnesiophyceae	*Phaeocystis*	3.5			Filtration <3 µm	Sorting		Big eukaryotes	K/100
872		MAR4	28	10	30 A2	Unknown	Unknown	3	+		Filtration <3 µm	Dilution			K/100
1001		MAR4	28	10	30 B	Prymnesiophyceae	*Emiliania*	3.5			Filtration <3 µm	Sorting		Small eukaryotes	K/100
1002		MAR4	28	10	31A	Prymnesiophyceae	*Phaeocystis?*	4.7	+		Filtration <3 µm	Sorting		Big eukaryotes	K/100
1003		MAR4	28	10	31 B	Unknown	Unknown	3			Filtration <3 µm	Sorting		Small eukaryotes	K/100
1004		MAR4	28	10	32 B	Prymnesiophyceae	Unknown	5			Filtration <3 µm	Sorting		Small eukaryotes	K/100
1048		MAR4	28	10	32 B2	Unknown	Unknown	3			Filtration <3 µm	Sorting		Eukaryotes	K/100
911		MAR4	28	10	32B_FL1-2	Prymnesiophyceae	*Emiliania*	3.5			Filtration <3 µm	Sorting	Serial dilution	Small eukaryotes	K/100
962		MAR4	28	10	32B_FL1-3	Prymnesiophyceae	*Emiliania*	3.5			Filtration <3 µm	Sorting	Serial dilution	Small eukaryotes	K/100
963		MAR4	28	10	32B_FL2-2	Unknown	Unknown	5			Filtration <3 µm	Sorting	Serial dilution	Small eukaryotes	K/100
955		MAR4	28	10	32B_HO22	Unknown	Unknown	3			Filtration <3 µm	Sorting	Micropipette	Small eukaryotes	K/100
920		MAR4	28	10	32B_HO3	Prymnesiophyceae	*Emiliania*	3.5			Filtration <3 µm	Sorting	Micropipette	Small eukaryotes	K/100
921		MAR4	28	10	32B_HO8	Prymnesiophyceae	*Emiliania*	3			Filtration <3 µm	Sorting	Micropipette	Small eukaryotes	K/100
1005	+	MAR4	28	10	33 A	Unknown	Unknown				Filtration <3 µm	Sorting		Eukaryotes	K/100
1049		MAR4	28	10	33 A2	Unknown	Unknown	2.5			Filtration <3 µm	Sorting		Eukaryotes	K/100
1006		MAR4	28	10	34 A	Prymnesiophyceae	*Phaeocystis*	3.5			Filtration <3 µm	Sorting		Big eukaryotes	K/100
854		MAR4	28	10	34 B2	Unknown	Unknown	2.5	+		Filtration <3 µm	Sorting		Big eukaryotes	K/100
956		MAR4	28	10	34B_HO16	Unknown	Unknown	3.5			Filtration <3 µm	Sorting	Micropipette	Eukaryotes	K/100
912		MAR4	28	10	34B_HO17	Prymnesiophyceae	*Emiliania*	4			Filtration <3 µm	Sorting	Micropipette	Eukaryotes	K/100
957		MAR4	28	10	34B_HO23	Unknown	Unknown	3.5			Filtration <3 µm	Sorting	Micropipette	Eukaryotes	K/100
914		MAR4	28	10	34B_HO5	Prymnesiophyceae	*Emiliania*	3			Filtration <3 µm	Sorting	Micropipette	Eukaryotes	K/100
958		MAR4	28	10	34B_HO6	Prymnesiophyceae	*Emiliania*	3.5			Filtration <3 µm	Sorting	Micropipette	Eukaryotes	K/100
916		MAR4	28	10	34B2_FL2-5	Prasinophyceae	*Prasinoderma*	5			Filtration <3 µm	Sorting	Serial dilution	Big eukaryotes	
1076		MAR4	28	10	37	Bicosoecid	*Caecitellus*	3.5		+	Filtration <3 µm				Rice/100
1007		MAR4	28	10	40 A	Dictyochophyceae	*Florenciella*	3.5			Filtration <0.6 µm	Sorting		Small eukaryotes	Pro2/100
857		MAR4	28	10	40 A2	Prasinophyceae	Unknown	2.5			Filtration <0.6 µm	Sorting		Big eukaryotes	Pro2/100
1008		MAR4	28	10	40 B	Dictyochophyceae	*Florenciella*	4			Filtration <0.6 µm	Sorting		Big eukaryotes	Pro2/100
855		MAR4	28	10	40 B2	Pelagophyceae	*Pelagomonas*	3.5			Filtration <0.6 µm	Sorting		Very big eukaryotes	Pro2/100
952		MAR4	28	10	41 A2	Unknown	Unknown	2.5			Filtration <0.6 µm	Dilution			Pro2/100
1009		MAR4	28	10	41 S	Unknown	Unknown	4			Filtration <0.6 µm	Sorting		Small eukaryotes	Pro2/100
856		MAR4	28	10	42 A2	Prasinophyceae	Unknown	2			Filtration <0.6 µm	Sorting		Small eukaryotes	Pro2/100
954		MAR4	28	10	43 A2	Unknown	Unknown	2.5			Filtration <0.6 µm	Dilution			Pro2/100
1010		MAR4	28	10	43 PK	Pelagophyceae	*Pelagomonas*	3			Filtration <0.6 µm	Sorting		*Prochlorococcus*	Pro2/100
953		MAR4	28	10	44 A2	Pelagophyceae	*Pelagomonas*	3			Filtration <0.6 µm	Dilution			Pro2/100
989		MAR4	28	60	15 A	Unknown	Unknown	3			Filtration <3 µm	Sorting		Small eukaryotes	K/100
982		MAR4	28	60	15 A2	Unknown	Unknown	2.5	+		Filtration <3 µm	Dilution			K/100
965		MAR4	28	60	16B_FL2-1	Bodonid	Unknown	5		+	Filtration <3 µm	Sorting	Serial dilution	*Prochlorococcus*	Pro2/100
983		MAR4	28	60	17 A2	Unknown	Unknown	2			Filtration <3 µm	Dilution			K/100
990		MAR4	28	60	17 B	Unknown	Unknown	2.5			Filtration <3 µm	Sorting		Small eukaryotes	K/100
991		MAR4	28	60	18 A	Unknown	Unknown	2.5			Filtration <3 µm	Sorting		Small eukaryotes	K/100
992		MAR4	28	60	18 B	Prymnesiophyceae	*Phaeocystis*	3.5			Filtration <3 µm	Sorting		Big eukaryotes	K/100
993		MAR4	28	60	19 B	Prymnesiophyceae	*Phaeocystis*	3			Filtration <3 µm	Sorting		Big eukaryotes	K/100
994		MAR4	28	60	19 C	Prymnesiophyceae	*Phaeocystis?*	5			Filtration <3 µm	Sorting		*Prochlorococcus*	K/100
1073	+	MAR4	28	60	22	Unknown	Unknown			+	Filtration <3 µm				Rice/100
984		MAR4	28	60	25 A2	Unknown	Unknown	2			Filtration <0.6 µm	Dilution			Pro2/100
1024		MAR4	28	60	25B2	Pelagophyceae	Unknown	4			Filtration <0.6 µm	Dilution			Pro2/100
985		MAR4	28	60	26 A2	Pelagophyceae	*Pelagomonas*	2			Filtration <0.6 µm	Dilution			Pro2/100
986		MAR4	28	60	27A2	Pelagophyceae	*Pelagomonas*	2.5			Filtration <0.6 µm	Dilution			Pro2/100
1043		HLN3	51	30	47 B1	Unknown	Unknown	3.5			Filtration <3 µm	Dilution			K/10
1011		HLN3	51	30	47C1S	Unknown	Unknown	2.5			Filtration <3 µm	Sorting		Small eukaryotes	K/100
852		HLN3	51	30	47 C2	Bolidophyceae	*Bolidomonas*	2			Filtration <3 µm	Dilution			K/100
1044	+	HLN3	51	30	47 C3	Unknown	Unknown				Filtration <3 µm	Dilution			K/100
1045	+	HLN3	51	30	47 D1	Unknown	Unknown				Filtration <3 µm	Dilution			K/100
1030		HLN3	51	30	48 A2Y	Cyanophyceae	*Synechococcus*	1			Filtration <0.6 µm	Sorting		*Synechococcus*	Pro2/10
850		HLN3	51	30	48 A5	Unknown	Unknown	2			Filtration <0.6 µm	Dilution			Pro2/10
1046		HLN3	51	30	48 A6	Unknown	Unknown	3			Filtration <0.6 µm	Dilution			Pro2/10
1027		HLN3	51	30	48 B3Y	Cyanophyceae	*Synechococcus*	1			Filtration <0.6 µm	Sorting		*Synechococcus*	Pro2/10
1012		HLN3	51	30	48 B6V	Unknown	Unknown	10			Filtration <0.6 µm	Sorting		Very big eukaryotes	Pro2/10
1031		HLN3	51	30	48 B6Y	Cyanophyceae	*Synechococcus*	1			Filtration <0.6 µm	Sorting		*Synechococcus*	Pro2/10

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Table 2. Continued.

RCC	Lost	Station	CTD	Depth	Preculture	Class	Genus	Size	Mixed	Hetereo-trophic	Step 1	Step 2	Step 3	Sorting target	Step 1 medium
1013		HLN3	51	30	48 C1S	Pelagophyceae	*Pelagomonas*	2			Filtration <0.6 µm	Sorting		Small eukaryotes	Pro2/100
858		HLN3	51	30	48 C3	Unknown	Unknown	2.5			Filtration <0.6 µm	Dilution			Pro2/100
1014		HLN3	51	30	48 D5V	Unknown	Unknown	2.5			Filtration <0.6 µm	Sorting		Very big eukaryotes	Pro2/100
880		HLN3	51	100	45 A2 475	Pelagophyceae	*Pelagomonas*	1.5			Filtration <3 µm	Dilution			K/10
1019		HLN3	51	100	45 A2 478	Unknown	Unknown	2.5			Filtration <3 µm	Dilution			K/10
995		HLN3	51	100	45 A2S	Pelagophyceae	*Pelagomonas*	2.5			Filtration <3 µm	Sorting		Small eukaryotes	K/10
881		HLN3	51	100	45 A3E	Pelagophyceae	*Pelagomonas*	2			Filtration <3 µm	Dilution			K/10
883		HLN3	51	100	45 A5	Pelagophyceae	*Pelagomonas*	2			Filtration <3 µm	Dilution			K/10
879		HLN3	51	100	45 B2E	Pelagophyceae	*Pelagomonas*	2.5			Filtration <3 µm	Sorting		Eukaryotes	K/10
1016		HLN3	51	100	45 B4 461	Cyanophyceae	*Synechococcus*	1			Filtration <3 µm	Dilution			K/10
1061		HLN3	51	100	45 B4 462	Pelagophyceae	*Pelagomonas*	2			Filtration <3 µm	Dilution			K/10
1017		HLN3	51	100	45 B5 463	Cyanophyceae	*Synechococcus*	1			Filtration <3 µm	Dilution			K/10
1062		HLN3	51	100	45 B5 464	Pelagophyceae	*Pelagomonas*	2.5			Filtration <3 µm	Dilution			K/10
1018		HLN3	51	100	45 B6 465	Cyanophyceae	*Synechococcus*	1			Filtration <3 µm	Dilution			K/10
884		HLN3	51	100	45 B6 466	Pelagophyceae	*Pelagomonas*	2.5			Filtration <3 µm	Dilution			K/10
1020		HLN3	51	100	45 C4Y	Cyanophyceae	*Synechococcus*	1			Filtration <3 µm	Sorting		*Synechococcus*	K/100
996		HLN3	51	100	46 B4S	Prasinophyceae	Unknown	3			Filtration <0.6 µm	Sorting		Small eukaryotes	Pro2/10
997		HLN3	51	100	46 B5S	Prasinophyceae	Unknown	2			Filtration <0.6 µm	Sorting		Small eukaryotes	Pro2/10
1021		HLN3	51	100	46 B6	Prasinophyceae	Unknown	4			Filtration <0.6 µm	Dilution			Pro2/10
1032		HLN3	51	100	46 B7	Prasinophyceae	Unknown	4			Filtration <0.6 µm	?			Pro2/10
998		HLN3	51	100	46 C3S	Prasinophyceae	Unknown	2.5			Filtration <0.6 µm	Sorting		Small eukaryotes	Pro2/100
999		HLN3	51	100	46 C4S 144	Prasinophyceae ?	Unknown	2.5			Filtration <0.6 µm	Sorting		Small eukaryotes	Pro2/100
859		GYR2	87	180	74 A5	Prasinophyceae	*Prasinococcus*	4			Sorting	Dilution		Small eukaryotes	K/100
853	+	GYR2	87	180	74 B1	Unknown stramenopile	Unknown	2.5			Sorting	Dilution		Small eukaryotes	K/100
1047	+	GYR2	87	180	74 B4	Unknown	Unknown	6			Sorting	Dilution		Small eukaryotes	K/100
863	+	GYR2	87	180	74 D5	Unknown	Unknown	3			Sorting	Dilution		Small eukaryotes	K/100
964		GYR2	87	300	70_FL1-1	Unknown	Unknown	2.5			Filtration <3 µm	Serial dilution			K/100
1065		GYR2	87	300	71	Bodonid	Unknown	4		+	Filtration <3 µm				Rice/100
1068		GYR2	87	300	72	Unknown	Unknown	3		+	Filtration <3 µm				Rice/100
987		STB11	121	200	79 A2	Unknown	Unknown	3	+		Filtration <3 µm	Dilution			K/100
1079		STB11	121	200	83	Bicosoecid	Unknown	3		+	Filtration <3 µm				Rice/100
862	+	STB12	125	40	90 A6	Unknown stramenopile	Unknown	2.5			Sorting	Dilution		Small eukaryotes	K
1066		STB13	129	0	105	Unknown	Unknown	3		+	Filtration <3 µm				Rice/100
968		STB13	129	160	92 B	Pelagophyceae	*Pelagomonas*	2.5			Filtration <3 µm	Sorting		Big eukaryotes	K/100
969		STB13	129	160	93 B	Pelagophyceae	*Pelagomonas*	2			Filtration <3 µm	Sorting		Big eukaryotes	K/100
1077		STB13	129	160	97	Bicosoecid	Unknown	3		+	Filtration <3 µm				Rice/100
988		STB13	129	160	98 A	Pelagophyceae	*Pelagomonas*	2.5			Filtration <3 µm	Dilution			Add Fe and P
866		STA14	132	5	108 B1	Prymnesiophyceae	Unknown	4	+		Sorting	Dilution		Small eukaryotes	K; K/100
971		STB14	133	150	109 A1	Pelagophyceae	*Pelagomonas*	2			Sorting	Dilution		Small eukaryotes	K; K/100
972		STB14	133	150	109 B1	Pelagophyceae	*Pelagomonas*	2			Sorting	Dilution		Big eukaryotes	K; K/100
973		STB14	133	150	109 B2	Pelagophyceae	*Pelagomonas*	2.5			Sorting	Dilution		Big eukaryotes	K; K/100
974		STB14	133	150	109 B3	Pelagophyceae	*Pelagomonas*	2.5			Sorting	Dilution		Big eukaryotes	K; K/100
1022		STB14	133	150	109 C2	Cyanophyceae	*Synechococcus*	1			Sorting	Dilution		*Synechococcus*	Pro2; Pro2/100
975		STB14	135	75	110 A1	Pelagophyceae	*Pelagomonas*	2.5			Sorting	Dilution		Very small eukaryotes	K; K/100
970		STB15	137	100	111 B1	Bicosoecid	*Cafeteria*	3		+	Sorting	Dilution		Small eukaryotes	K; K/100
980		STB15	137	100	111 B2	Pelagophyceae	*Pelagomonas*	2			Sorting	Dilution		Small eukaryotes	K; K/100
981		STB15	137	100	111 C1E	Pelagophyceae	*Pelagomonas*	2			Sorting	Dilution		Big eukaryotes	K; K/100
978		STB15	137	100	111 D1E	Pelagophyceae	*Pelagomonas*	2	+		Sorting	Dilution		Big eukaryotes	K; K/100
1023		STB15	137	100	112 B6	Cyanophyceae	*Synechococcus*	1			Sorting	Dilution		*Synechococcus*	Pro2; Pro2/100
869		EGY2	146	5	121 A	Pelagophyceae	*Pelagomonas*	2.5			Filtration <0.6 µm	Sorting		Small eukaryotes	K/100
870	+	EGY2	146	5	121 B	Prymnesiophyceae	*Phaeocystis*	5			Filtration <0.6 µm	Sorting		Big eukaryotes	K/100
938		EGY2	146	5	122 A	Pelagophyceae	*Pelagomonas*	2			Filtration <0.6 µm	Sorting		Small eukaryotes	K/100
940		EGY2	146	5	122 B	Prymnesiophyceae	*Phaeocystis*	5			Filtration <0.6 µm	Sorting		Big eukaryotes	K/100
868		EGY2	146	5	122 C	Prymnesiophyceae	*Emiliania*	4			Filtration <0.6 µm	Sorting		Big eukaryotes	K/100
1051		EGY2	146	5	123 A	Unknown	Unknown	3			Filtration <0.6 µm	Sorting		Small eukaryotes	Pro2/100
939		EGY2	146	5	123 B	Pelagophyceae	*Pelagomonas*	3			Filtration <0.6 µm	Sorting		Big eukaryotes	Pro2/100
1072		EGY2	146	5	125	Bicosoecid	*Caecitellus*	3.5		+	Filtration <0.6 µm				Rice/100
1078		EGY2	146	5	126	Bicosoecid	*Caecitellus*	3.5		+	Filtration <0.6 µm				Rice/100
1052		EGY2	146	5	129 A1 545	Unknown	Unknown	2.5			Sorting	Dilution		Very small eukaryotes	K; K/100
1053		EGY2	146	5	129 A2	Unknown	Unknown	2.5			Sorting	Dilution		Very small eukaryotes	K; K/100
864		EGY2	146	5	129 A3	Unknown	Unknown	2			Sorting	Dilution		Very small eukaryotes	K; K/100
860		EGY2	146	5	129 B1	Pelagophyceae	*Pelagomonas*	2			Sorting	Dilution		Very small eukaryotes	K; K/100
1035		EGY2	146	5	129 B2	Unknown	Unknown	2.5			Sorting	Dilution		Very small eukaryotes	K; K/100
1036		EGY2	146	5	129 B3 661	Unknown	Unknown	3	+		Sorting	Dilution		Very small eukaryotes	K; K/100
861		EGY2	146	5	129 C1 552	Prymnesiophyceae	*Phaeocystis*	3			Sorting	Dilution		Small eukaryotes	K; K/100
1037		EGY2	146	5	129 C1 662	Unknown	Unknown	2.5			Sorting	Dilution		Small eukaryotes	K; K/100
865		EGY2	146	5	129 C2B	Pelagophyceae	*Pelagomonas*	3			Sorting	Sorting		Eukaryotes	K; K/100
867		EGY2	146	5	130 A1	Prymnesiophyceae	*Emiliania*	5			Sorting	Dilution		Eukaryotes	K; K/100
874		EGY2	146	5	130 A1BC	Unknown	Unknown	4			Sorting	Sorting		Eukaryotes	K; K/100
875		EGY2	146	5	130 A1E	Pelagophyceae	*Pelagomonas*	3			Sorting	Sorting		Eukaryotes	K; K/100

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Table 2. Continued.

RCC	Lost	Station	CTD	Depth	Preculture	Class	Genus	Size	Mixed	Hetereo-trophic	Step 1	Step 2	Step 3	Sorting target	Step 1 medium
1071		EGY2	146	80	118	Bicosoecid	*Cafeteria*	3		+	Filtration <0.6 µm				Rice/100
976		EGY3	154	80	131 A1	Pelagophyceae	*Pelagomonas*	2.5			Sorting	Dilution		Small eukaryotes	K; K/100
979		EGY3	154	80	131 A3	Pelagophyceae	*Pelagomonas*	2			Sorting	Dilution		Small eukaryotes	K; K/100
977		EGY3	154	80	131 B1	Pelagophyceae	*Pelagomonas*	2.5			Sorting	Dilution		Big eukaryotes	K; K/100
873		EGY4	162	40	140 A	Unknown	Unknown	2			Filtration <0.6 µm	Sorting		Small eukaryotes	K/100
951		EGY4	162	40	140 B	Prymnesiophyceae	*Emiliania*	3.5			Filtration <0.6 µm	Sorting		Eukaryotes	K/100
871		EGY4	162	40	140 C	Pelagophyceae	*Pelagomonas*	2.5			Filtration < 0.6 µm	Sorting		Big eukaryotes	K/100
1050		EGY4	162	40	141 A	Unknown	Unknown	2.5			Filtration <0.6 µm	Sorting		Small eukaryotes	K/100
937		EGY4	162	40	141 B	Pelagophyceae	*Pelagomonas*	3			Filtration <0.6 µm	Sorting		Big eukaryotes	K/100
1029		EGY4	162	40	141 D	Cyanophyceae	*Synechococcus*	1			Filtration <0.6 µm	Sorting		*Synechococcus*	K/100
1069		EGY4	162	40	145	Bicosoecid	Unknown	5		+	Filtration <0.6 µm				Rice/100
1054		EGY4	162	40	148 A1C1-1	Unknown	Unknown	5			Sorting	Dilution	Plating	Small eukaryotes	K; K/100
1055		EGY4	162	40	148 A1C1-2	Unknown	Unknown	7			Sorting	Dilution	Plating	Small eukaryotes	K; K/100
878		EGY4	162	40	148 B1 602	Unknown	Unknown	3	+		Sorting	Dilution		Big eukaryotes	K; K/100
876		EGY4	162	40	148 B2 600	Unknown	Unknown	4			Sorting	Dilution		Big eukaryotes	K; K/100
877		EGY4	162	40	148 B2E	Unknown	Unknown	3	+		Sorting	Sorting		Big eukaryotes	K; K/100
1028		EGY4	162	40	148 D3	Cyanophyceae	*Synechococcus*	1			Sorting	Dilution		*Synechococcus*	Pro2; Pro2/100
1070		EGY4	162	80	136	Unknown	Unknown	4	+		Filtration <0.6 µm				Rice/100
1081	+	EGY4	162	80	137	Unknown	Unknown			+	Filtration <0.6 µm				Rice/100
1038		STB17	178	20	150	Unknown	Unknown	3		+	Sorting			Very small eukaryotes	K/10
1039		STB17	178	20	153	Unknown	Unknown	3		+	Sorting			Big eukaryotes	K/10
848		STB17	178	20	158	Dinophyceae	*Prorocentrum*	15			Sorting			High red & green fluorescencing	K/10
1040		STB20	190	5	184	Unknown	Unknown	3		+	Sorting			Very small eukaryotes	K/10
1041	+	STB20	190	5	185	Unknown	Unknown			+	Sorting			Small eukaryotes	K/10
909		STB20	191	5	179_FL1-2	Unknown	Unknown	4	+		Filtration <0.6 µm	Serial dilution			Pro2/100
919		STB20	191	5	179_FL1-3	Unknown	Unknown	6			Filtration <0.6 µm	Serial dilution			Pro2/100
918		STB20	191	5	179_FL2-1	Unknown	Unknown	3.5	+		Filtration <0.6 µm	Serial dilution			Pro2/100
1064		STB20	191	5	181	Bicosoecid	*Caecitellus*	2		+	Filtration <0.6 µm				Rice/100
922		STB20	191	5	182_FL1-1	Dinophyceae	*Prorocentrum*	14			Filtration <0.6 µm	Serial dilution			Add Fe and P
917		STB20	191	5	182_FL1-3	Prasinophyceae	Unknown	1.5	+		Filtration <0.6 µm	Serial dilution			Add Fe and P
928		STB20	191	40	169_FL2-2	Unknown	Unknown	3			Filtration <0.6 µm	Serial dilution			K/100
941		STB20	191	40	169_FL2-4	Prasinophyceae	*Prasinoderma ?*	3.5			Filtration <0.6 µm	Serial dilution			K/100
1075	+	STB20	191	40	173	Unknown	Unknown			+	Filtration <0.6 µm				Rice/100
948		STB20	191	40	175_FL2-4	Prymnesiophyceae	*Emiliania*	3.5			Filtration <0.6 µm	Serial dilution			Add Fe and P
1042		STB21	194	5	189	Unknown	Unknown	3			Sorting			Big eukaryotes	K/10
1056		STB21	194	5	190 C2-1	Unknown	Unknown	3	+		Sorting	Plating		Green, yellow and red fluorescencing	K/10
1057		STB21	194	5	190 C2-2	Unknown	Unknown	4	+		Sorting	Plating		Green, yellow and red fluorescencing	K/10
910		STB21	194	5	190_FL2-4	Prasinophyceae	*Prasinoderma ?*	4			Sorting	Serial dilution		Green, yellow and red fluorescencing	K/10
849		UPW1	198	5	193	Prymnesiophyceae	*Phaeocystis*	3	+		Sorting			Eukaryotes	K/10
1025		UPW1	198	5	194	Bacillariophyceae	*Chaetoceros*	15			Sorting			*Synechococcus*	Pro2/10
1026		UPW1	198	5	199	Cyanophyceae	*Synechococcus*	1			Sorting			*Synechococcus*	Pro2/10
851		UPW1	198	5	202	Prymnesiophyceae	*Phaeocystis*	4			Sorting			Very big eukaryotes	K/10
924		UPW3	210	5	221_FL1-1	Unknown	Unknown	2	+		Filtration <0.6 µm	Serial dilution			K/100
915		UPW3	210	5	223_FL2-3	Pelagophyceae	*Pelagomonas*	4			Filtration <0.6 µm	Serial dilution			Pro2/100
925		UPW3	210	5	224_FL2-3	Prymnesiophyceae	*Phaeocystis*	5			Filtration <0.6 µm	Serial dilution			Rice/100
908		UPW3	210	5	226_FL2-3	Prymnesiophyceae	*Phaeocystis*	5	+		Filtration <0.6 µm	Serial dilution			Add Fe and P
1058		UPW3	210	30	206	Prasinophyceae	*Pycnococcus*	2.5			Sorting			Very small eukaryotes	K/10
931		UPW3	210	30	206_FL1-1	Prasinophyceae	*Pycnococcus*	1.7			Sorting	Serial dilution		Very small eukaryotes	K/10
882		UPW3	210	30	208	Prymnesiophyceae	*Phaeocystis*	5			Sorting			Big eukaryotes	K/10
1033		UPW3	210	30	208_FL2-6	Prymnesiophyceae	*Phaeocystis*	10			Sorting	Serial dilution		Big eukaryotes	K/10
1059		UPW3	210	30	209	Prasinophyceae	*Pycnococcus*	2			Sorting			Orange fluorescencing	K/10
1015		UPW3	210	30	211	Cyanophyceae	*Synechococcus*	1			Sorting			Big cyanobacteria	Pro2/10
932		UPW3	210	30	212_FL1-2	Prasinophyceae	*Pycnococcus*	4	+		Filtration <0.6 µm	Serial dilution			K/100
930		UPW3	210	30	212_FLA2	Prasinophyceae	*Prasinoderma ?*	3			Filtration <0.6 µm	Micropipette			K/100
936		UPW3	210	30	212_FLA5	Prasinophyceae	*Prasinoderma ?*	2.2			Filtration <0.6 µm	Micropipette			K/100
934		UPW3	210	30	214_FLB3	Prasinophyceae	*Prasinoderma?*	2.5			Filtration <0.6 µm	Micropipette			Pro2/100
933		UPW3	210	30	214_FLB6	Prasinophyceae	*Prasinoderma?*	2.5			Filtration <0.6 µm	Micropipette			Pro2/100
1080		UPW3	210	30	216	Unknown	Unknown	3.5		+	Filtration <0.6 µm				Rice/100
929		UPW3	210	30	219_FL1-4	Prasinophyceae	*Prasinoderma?*	2.5			Filtration <0.6 µm	Serial dilution			Add Fe and P
935		UPW3	210	30	219_FL2-3	Prymnesiophyceae	*Phaeocystis*	5			Filtration <0.6 µm	Serial dilution			Add Fe and P
947		UPW3	210	30	219_FLC3	Unknown	Unknown	4	+		Filtration <0.6 µm	Micropipette			Add Fe and P
946		UPX	213	0	235_FL1-4	Prasinophyceae	*Prasinoderma?*	3			Filtration <0.6 µm	Serial dilution			Add Fe and P
926		UPX	213	0	235_FL2-3	Pelagophyceae	*Pelagomonas*	2.5			Filtration <0.6 µm	Serial dilution			Add Fe and P
950		UPX	213	0	237_DVA4	Bacillariophyceae	*Thalassiosira*	13			Filtration <0.6 µm	Serial dilution			K/100
943		UPX	213	0	237_DVB3	Pelagophyceae	*Pelagomonas*	3			Filtration <0.6 µm	Serial dilution			K/100
945		UPX	213	0	237_DVB4	Trebouxiophyceae	*Picochlorum*	2			Filtration <0.6 µm	Serial dilution			K/100
944		UPX	213	0	237_DVC4	Trebouxiophyceae	*Picochlorum*	1.5			Filtration <0.6 µm	Serial dilution			K/100

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Table 2. Continued.

RCC	Lost	Station	CTD	Depth	Preculture	Class	Genus	Size	Mixed	Hetereo-trophic	Step 1	Step 2	Step 3	Sorting target	Step 1 medium
1034		UPX	213	0	237_DVE6	Trebouxiophyceae	*Picochlorum*	1.5			Filtration <0.6 μm	Serial dilution			K/100
949		UPX	213	0	242_DVA9	Unknown	Unknown	2.5			Filtration <0.6 μm	Serial dilution			Add Fe and P
927		UPX	213	0	243_FL1-4	Prasinophyceae	*Prasinoderma?*	3			Filtration <0.6 μm	Serial dilution			Add Fe and P
942		UPX	213	40	231_FL1-2	Unknown	Unknown	4	+		Filtration <0.6 μm	Serial dilution			Pro2/100
1063		UPX	213	40	232	Bicosoecid	Unknown	3.5		+	Filtration <0.6 μm				Rice/100
1074		UPX	213	40	233	Unknown	Unknown	3		+	Filtration <0.6 μm				Rice/100
966		UPX	213	40	234_DVD10	Prasinophyceae	*Micromonas*	1.5	+		Filtration <0.6 μm	Serial dilution			Add Fe and P
913		UPX	213	40	234_DVD11	Prasinophyceae	*Micromonas*	2			Filtration <0.6 μm	Serial dilution			Add Fe and P
967		UPX	213	40	234_DVH12	Bacillariophyceae	*Minutocellus*	3	+		Filtration <0.6 μm	Serial dilution			Add Fe and P
1060		UPX	213	40	244	Prasinophyceae	*Pycnococcus*	2			Sorting			Big eukaryotes	K/10
1067		UPX	213	40	248	Unknown	Unknown	20		+	Sorting			*Synechococcus*	Pro2/10

Table 3. List of identified cultures ordered by phylogenetic group. The column rDNA cluster provides the RCC number of the reference culture for each cluster defined using Fast Group II (http://biome.sdsu.edu/fastgroup/fg_tools.htm) with the parameter sequence match set at 80%. For cultures for which no sequence was obtained this column remains empty.

Division	Class	Order	Genus	RCC	rRNA cluster	Preculture	Station	Depth	Size (μm)	Cell shape	Assemblage	Hetero- trophic	Lost
Cyanophyta	Cyanophyceae		*Synechococcus*	1031		48 B6Y	HLN3	30	1.0	cylindrical			
Cyanophyta	Cyanophyceae		*Synechococcus*	1030		48 A2Y	HLN3	30	1.0	cylindrical			
Cyanophyta	Cyanophyceae		*Synechococcus*	1027		48 B3Y	HLN3	30	1.0	cylindrical	chains		
Cyanophyta	Cyanophyceae		*Synechococcus*	1017		45 B5 463	HLN3	100	1.0	cylindrical	chains		
Cyanophyta	Cyanophyceae		*Synechococcus*	1018		45 B6 465	HLN3	100	1.0	cylindrical	chains		
Cyanophyta	Cyanophyceae		*Synechococcus*	1020		45 C4Y	HLN3	100	1.0	cylindrical	chains		
Cyanophyta	Cyanophyceae		*Synechococcus*	1016		45 B4 461	HLN3	100	1.0	elongated	chains		
Cyanophyta	Cyanophyceae		*Synechococcus*	1022		109 C2	STB14	150	1.0	cylindrical			
Cyanophyta	Cyanophyceae		*Synechococcus*	1023		112 B6	STB15	100	1.0	cylindrical			
Cyanophyta	Cyanophyceae		*Synechococcus*	1028		148 D3	EGY4	40	1.0	cylindrical			
Cyanophyta	Cyanophyceae		*Synechococcus*	1029		141 D	EGY4	40	1.0	coccoid			
Cyanophyta	Cyanophyceae		*Synechococcus*	1026		199	UPW1	5	1.0	cylindrical	chains		
Cyanophyta	Cyanophyceae		*Synechococcus*	1015		211	UPW3	30	1.0	cylindrical			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma*	907	916	271_FL1-4	MAR3	60	3.5	coccoid			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma*	916	916	34B2_FL2-5	MAR4	10	5.0	coccoid			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma?*	941	927	169_FL2-4	STB20	40	3.5	coccoid			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma?*	910	927	190_FL2-4	STB21	5	4.0	coccoid			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma?*	936	927	212_FLA5	UPW3	30	2.2	coccoid			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma?*	934	927	214_FLB3	UPW3	30	2.5	coccoid			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma?*	933	927	214_FLB6	UPW3	30	2.5	coccoid			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma?*	929	927	219_FL1-4	UPW3	30	2.5	oval			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma ?*	930	927	212_FLA2	UPW3	30	3.0	coccoid			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma ?*	946	927	235_FL1-4	UPX	0	3.0	flagellate			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinoderma ?*	927	927	243_FL1-4	UPX	0	3.0	coccoid			
Chlorophyta	Prasinophyceae	Prasinococcales	*Prasinococcus*	859	859	74 A5	GYR2	180	4.0	coccoid surrounded by matrix			
Chlorophyta	Prasinophyceae	Pseudoscourfieldiales	*Pycnococcus*	931	932	206_FL1-1	UPW3	30	1.7	coccoid			
Chlorophyta	Prasinophyceae	Pseudoscourfieldiales	*Pycnococcus*	1059	932	209	UPW3	30	2.0	coccoid	clumps		
Chlorophyta	Prasinophyceae	Pseudoscourfieldiales	*Pycnococcus*	1058	932	206	UPW3	30	2.5	coccoid			
Chlorophyta	Prasinophyceae	Pseudoscourfieldiales	*Pycnococcus*	932	932	212_FL1-2	UPW3	30	4.0	coccoid	clumps		
Chlorophyta	Prasinophyceae	Pseudoscourfieldiales	*Pycnococcus*	1060	932	244	UPX	40	2.0	coccoid	clumps		
Chlorophyta	Prasinophyceae	clade VIIA	Unknown	857	917	40 A2	MAR4	10	2.5	coccoid			
Chlorophyta	Prasinophyceae	clade VIIA	Unknown	856	917	42 A2	MAR4	10	2.0	coccoid			
Chlorophyta	Prasinophyceae	clade VIIA	Unknown	996	917	46 B4S	HLN3	100	3.0	coccoid			
Chlorophyta	Prasinophyceae	clade VIIA	Unknown	997	997	46 B5S	HLN3	100	2.0	coccoid			
Chlorophyta	Prasinophyceae	clade VIIA	Unknown	1021	917	46 B6	HLN3	100	4.0	coccoid			
Chlorophyta	Prasinophyceae	clade VIIA	Unknown	1032	917	46 B7	HLN3	100	4.0	coccoid			
Chlorophyta	Prasinophyceae	clade VIIA	Unknown	998	998	46 C3S	HLN3	100	2.5	coccoid			
Chlorophyta	Prasinophyceae	clade VIIA	Unknown	917	917	182_FL1-3	STB20	5	1.5	coccoid			
Chlorophyta	Prasinophyceae	Mamiellales	*Micromonas*	966	913	234_DVD10	UPX	40	1.5	flagellate			
Chlorophyta	Prasinophyceae	Mamiellales	*Micromonas*	913	913	234_DVD11	UPX	40	2.0	flagellate			
Chlorophyta	Prasinophyceae?		Unknown	999	999	46 C4S 144	HLN3	100	2.5	coccoid			
Chlorophyta	Trebouxiophyceae		*Picochlorum*	944	945	237_DVC4	UPX	0	1.5	oval			
Chlorophyta	Trebouxiophyceae		*Picochlorum*	1034	945	237_DVE6	UPX	0	1.5	elongated			
Chlorophyta	Trebouxiophyceae		*Picochlorum*	945	945	237_DVB4	UPX	0	2.0	oval			
Haptophyta	Prymnesiophyceae		*Phaeocystis*	1000	940	30 A	MAR4	10	3.5	flagellate			
Haptophyta	Prymnesiophyceae		*Phaeocystis*	1006	940	34 A	MAR4	10	3.5	flagellate			

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Table 3. Continued.

Division	Class	Order	Genus	RCC	rRNA cluster	Preculture	Station	Depth	Size (μm)	Cell shape	Assemblage	Hetero- trophic	Lost
Haptophyta	Prymnesiophyceae		*Phaeocystis*	993	940	19 B	MAR4	60	3.0	flagellate			
Haptophyta	Prymnesiophyceae		*Phaeocystis*	992	940	18 B	MAR4	60	3.5	flagellate			
Haptophyta	Prymnesiophyceae		*Phaeocystis*	861	940	129 C1 552	EGY2	5	3.0	flagellate			
Haptophyta	Prymnesiophyceae		*Phaeocystis*	870	940	121 B	EGY2	5	5.0	coccoid	colonies		+
Haptophyta	Prymnesiophyceae		*Phaeocystis*	940	940	122 B	EGY2	5	5.0	coccoid			
Haptophyta	Prymnesiophyceae		*Phaeocystis*	849	849	193	UPW1	5	3.0	flagellate	colonies		
Haptophyta	Prymnesiophyceae		*Phaeocystis*	851	940	202	UPW1	5	4.0	coccoid	colonies		
Haptophyta	Prymnesiophyceae		*Phaeocystis*	882	940	208	UPW3	30	5.0	coccoid			
Haptophyta	Prymnesiophyceae		*Phaeocystis*	1033	940	208_FL2-6	UPW3	30	10.0	coccoid			
Haptophyta	Prymnesiophyceae		*Phaeocystis?*	1002		31 A	MAR4	10	4.7	flagellate			
Haptophyta	Prymnesiophyceae		*Phaeocystis?*	994		19 C	MAR4	60	5.0	flagellate+ coccoid			
Haptophyta	Prymnesiophyceae		*Phaeocystis*	908	940	226_FL2-3	UPW3	5	5.0	elongated			
Haptophyta	Prymnesiophyceae		*Phaeocystis*	925	940	224_FL2-3	UPW3	5	5.0	coccoid			
Haptophyta	Prymnesiophyceae		*Phaeocystis*	935	912	219_FL2-3	UPW3	30	5.0	flagellate			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	914	912	34B_HO5	MAR4	10	3.0	coccoid			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	921	912	32B_HO8	MAR4	10	3.0	coccoid			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	962	912	32B_FL1-3	MAR4	10	3.5	round			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	920	912	32B_HO3	MAR4	10	3.5	coccoid			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	911	912	32B_FL1-2	MAR4	10	3.5	coccoid			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	958	912	34B_HO6	MAR4	10	3.5	coccoid			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	1001	912	30 B	MAR4	10	3.5	coccoid			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	912	912	34B_HO17	MAR4	10	4.0	coccoid			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	868	912	122 C	EGY2	5	4.0	coccoid			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	867	912	130 A1	EGY2	5	5.0	coccoid			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	951	912	140 B	EGY4	40	3.5	coccoid			
Haptophyta	Prymnesiophyceae	Isochrysidales	*Emiliania*	948	912	175_FL2-4	STB20	40	3.5	round			
Haptophyta	Prymnesiophyceae	Coccolithophorales	Unknown	1004		32 B	MAR4	10	5.0	round			
Haptophyta	Prymnesiophyceae	Coccolithophorales	Unknown	866		108 B1	STA14	5	4.0	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	953	926	44 A2	MAR4	10	3.0	elongated			
Stramenopile	Pelagophyceae		*Pelagomonas*	1010	926	43 PK	MAR4	10	3.0	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	855	926	40 B2	MAR4	10	3.5	elongated			
Stramenopile	Pelagophyceae		*Pelagomonas*	985	926	26 A2	MAR4	60	2.0	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	1013	926	48 C1S	HLN3	30	2.0	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	880	926	45 A2 475	HLN3	100	1.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	883	926	45 A5	HLN3	100	2.0	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	881	926	45 A3E	HLN3	100	2.0	bean-shaped			
Stramenopile	Pelagophyceae		*Pelagomonas*	1061	926	45 B4 462	HLN3	100	2.0	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	995	926	45 A2S	HLN3	100	2.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	1062	926	45 B5 464	HLN3	100	2.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	884	926	45 B6 466	HLN3	100	2.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	879	926	45 B2E	HLN3	100	2.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	969	926	93 B	STB13	160	2.0	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	988	926	98 A	STB13	160	2.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	968	926	92 B	STB13	160	2.5	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	972	926	109 B1	STB14	150	2.0	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	971	926	109 A1	STB14	150	2.0	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	974	926	109 B3	STB14	150	2.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	973	926	109 B2	STB14	150	2.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	975	926	110 A1	STB14	75	2.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	981	926	111 C1E	STB15	100	2.0	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	980	926	111 B2	STB15	100	2.0	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	978	978	111 D1E	STB15	100	2.0	flagellate			

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Table 3. Continued.

Division	Class	Order	Genus	RCC	rRNA cluster	Preculture	Station	Depth	Size (μm)	Cell shape	Assemblage	Hetero- trophic	Lost
Stramenopile	Pelagophyceae		*Pelagomonas*	938	926	122 A	EGY2	5	2.0	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	860	926	129 B1	EGY2	5	2.0	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	869	926	121 A	EGY2	5	2.5	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	939	926	123 B	EGY2	5	3.0	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	875	926	130 A1E	EGY2	5	3.0	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	865	926	129 C2B	EGY2	5	3.0	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	979	926	131 A3	EGY3	80	2.0	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	976	926	131 A1	EGY3	80	2.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	977	926	131 B1	EGY3	80	2.5	flagellate			
Stramenopile	Pelagophyceae		*Pelagomonas*	871	926	140 C	EGY4	40	2.5	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	937	926	141 B	EGY4	40	3.0	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	915	926	223_FL2-3	UPW3	5	4.0	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	926	926	235_FL2-3	UPX	0	2.5	coccoid			
Stramenopile	Pelagophyceae		*Pelagomonas*	943	926	237_DVB3	UPX	0	3.0	coccoid			
Stramenopile	Pelagophyceae		Unknown	1024	926	25 B2	MAR4	60	4.0	round			
Stramenopile	Pelagophyceae		Unknown	986	926	27 A2	MAR4	60	2.5	coccoid			
Stramenopile	Bolidophyceae		*Bolidomonas*	852	852	47 C2	HLN3	30	2.0	flagellate			
Stramenopile	Dictyochophyceae		*Florenciella*	1007	1007	40 A	MAR4	10	3.5	flagellate			
Stramenopile	Dictyochophyceae		*Florenciella*	1008	1007	40 B	MAR4	10	4.0	flagellate			
Stramenopile	Unknown		Unknown	853	853	74 B1	GYR2	180	2.5	coccoid			
Stramenopile	Unknown		Unknown	862	853	90 A6	STB12	40	2.5	coccoid			
Stramenopile	Bacillariophyceae		*Chaetoceros*	1025		194	UPW1	5	15.0	rectangular	chains		
Stramenopile	Bacillariophyceae		*Minutocellus*	967	967	234_DVH12	UPX	40	3.0	rectangular			
Stramenopile	Bacillariophyceae		*Thalassiosira*	950	950	237_DVA4	UPX	0	13.0	elongated			
Alveolata	Dinophyceae		*Prorocentrum*	848	848	158	STB17	20	15.0	irregular			
Alveolata	Dinophyceae		*Prorocentrum*	922		182_FL1-1	STB20	5	14.0	flagellate			
Stramenopile	Bicosoecid		*Caecitellus*	1076	1072	37	MAR4	10	3.5	flagellate		+	
Stramenopile	Bicosoecid		*Caecitellus*	1072	1072	125	EGY2	5	3.5	flagellate		+	
Stramenopile	Bicosoecid		*Caecitellus*	1078	1072	126	EGY2	5	3.5	flagellate		+	
Stramenopile	Bicosoecid		*Caecitellus*	1064	1072	181	STB20	5	2.0	flagellate		+	
Stramenopile	Bicosoecid		*Cafeteria*	970	1077	111 B1	STB15	100	3.0	flagellate		+	
Stramenopile	Bicosoecid		*Cafeteria*	1071	1077	118	EGY2	80	3.0	flagellate		+	
Stramenopile	Bicosoecid		*Cafeteria*	1077	1077	97	STB13	160	3.0	flagellate		+	
Stramenopile	Bicosoecid		Unknown	1079	1079	83	STB11	200	3.0	flagellate		+	
Stramenopile	Bicosoecid		Unknown	1069	1069	145	EGY4	40	5.0	flagellate		+	
Stramenopile	Bicosoecid		Unknown	1063	1063	232	UPX	40	3.5	flagellate		+	
Kinetoplastida	Bodonid		Unknown	965	965	16B_FL2-1	MAR4	60	5.0	flagellate		+	
Kinetoplastida	Bodonid		Unknown	1065	1065	71	GYR2	300	4.0	flagellate		+	

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Table 4. Number of strains identified for the different phylogenetic groups.

Division	Class	Genus	Number	Total per division
Cyanobacteria	Cyanophyceae	*Synechococcus*	13	13
Chlorophyta	Prasinophyceae	*Micromonas*	2	31
	Prasinophyceae	*Prasinococcus*	1	
	Prasinophyceae	*Prasinoderma*	2	
	Prasinophyceae	*Prasinoderma ?*	9	
	Prasinophyceae	*Pycnococcus*	5	
	Prasinophyceae	Unknown	9	
	Trebouxiophyceae	*Picochlorum*	3	
Stramenopiles	Bacillariophyceae	*Chaetoceros*	1	58
	Bacillariophyceae	*Minutocellus*	1	
	Bacillariophyceae	*Thalassiosira*	1	
	Bolidophyceae	*Bolidomonas*	1	
	Dictyochophyceae	*Florenciella*	2	
	Pelagophyceae	*Pelagomonas*	38	
	Pelagophyceae	Unknown	2	
	Unknown	Unknown	2	
	Bicosoecid	*Caecitellus*	4	
	Bicosoecid	*Cafeteria*	3	
	Bicosoecid	Unknown	3	
Haptophyta	Prymnesiophyceae	*Emiliania*	12	30
	Prymnesiophyceae	*Phaeocystis*	14	
	Prymnesiophyceae	*Phaeocystis?*	2	
	Prymnesiophyceae	Unknown	2	
Alveolata	Dinophyceae	*Prorocentrum*	2	2
Kinetoplastida	Bodonid	Unknown	2	

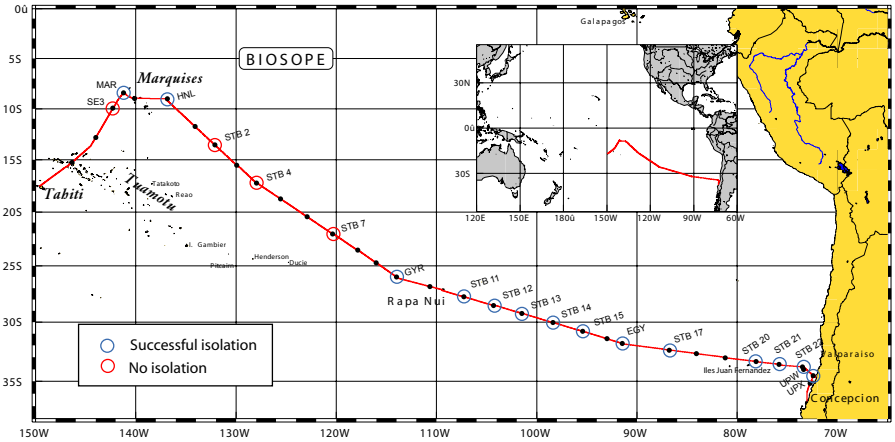

Fig. 1. BIOSOPE cruise track displaying the location of stations sampled for cultures.

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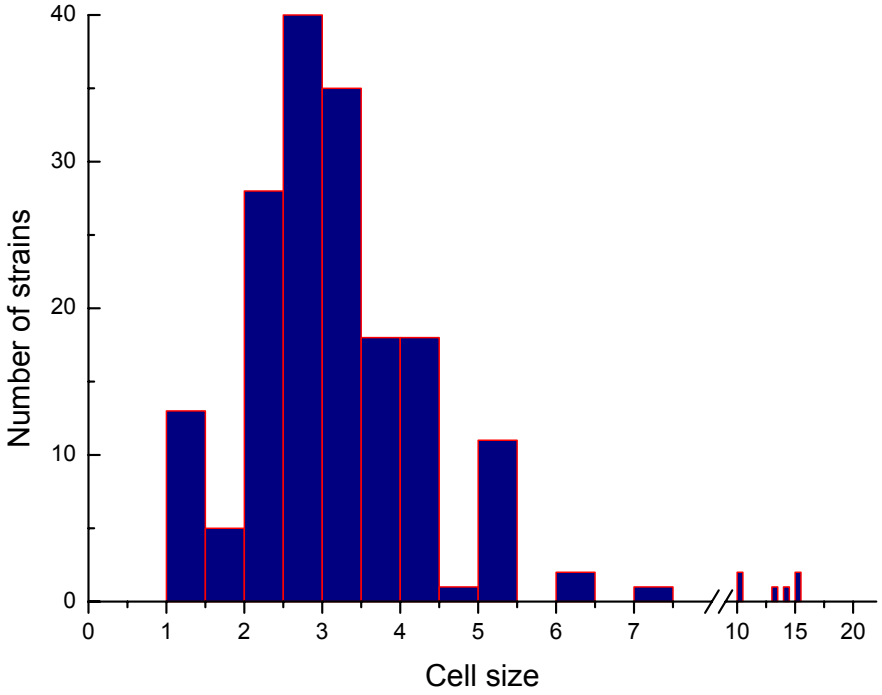

Fig. 2. Size histogram of all RCC cultures recovered from the BIOSOPE cruise.

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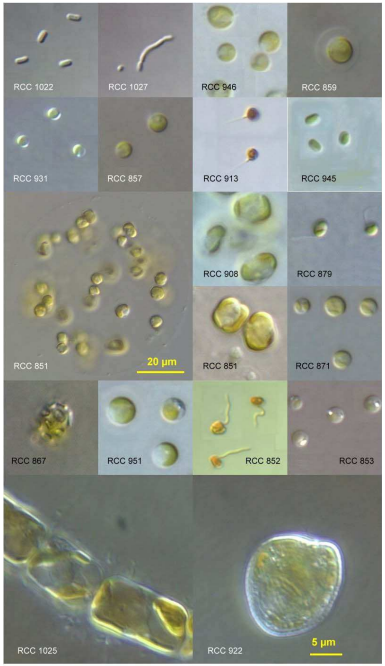

Fig. 3. Microscopy images of a selection of strains recovered during the BIOSOPE cruise. Scale bar is 5 μm for all images except for the *Phaeocystis* colony (RCC 851). From top to bottom and from left to right. Cyanobacteria: RCC 1022 and 1027. Note the elongated shape and short chains made by RCC 1027. Prasinophyceae: *Prasinoderma* sp. (RCC 946), *Prasinococcus capsulatus* (RCC 859), *Pycnococcus provasoli* (RCC 931), undescribed species belonging to clade VII (RCC 857) and *Micromonas pusilla* (RCC 913). Trebouxiophyceae: *Picochlorum* sp. (RCC 945). Prymnesiophyceae: *Phaeocystis* sp. (RCC 851 and 908, note colonial form) and *Emiliana huxleyi* (RCC 867, calcifying, and RCC 951, not calcifying). Pelagophyceae: *Pelagomonas calceolata* (RCC 879, flagellated lugol fixed, and RCC 871, coccoid). Bolidophyceae: *Bolidomonas* sp. (RCC 852, lugol fixed). Heterokontophyta: unknown species (RCC 853). Diatom: *Chaetoceros* sp. (RCC 1025). Dinoflagellate: *Prorocentrum minimum* (RCC 922).

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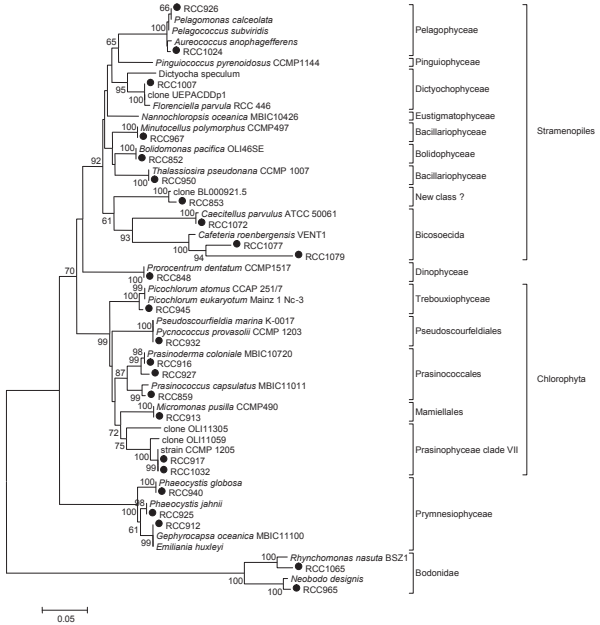

Fig. 4. Phylogenetic analysis of selected strains recovered during the BIOSOPE cruise. One or two 18 S rRNA sequences from each taxonomic group was selected following clustering with Fast Group II (see Material and Methods). Neighbour-joining optimal tree with the sum of branch length=1.73998869 shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are displayed next to the branches. Only values larger than 60% are shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 394 positions in the final dataset. Phylogenetic analyses were conducted with MEGA4 (http://www.megasoftware.net/mega4/).

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